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# Early-Infantile Galactosialidosis: Clinical, Biochemical, and Molecular Observations in a New Patient

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**Few patients with the early-infantile form of galactosialidosis have been described to date. Presented here is the first Italian case. Fetal hydrops was detected by ultrasound at week 24 of gestation. At birth, the infant presented with hypotonia, massive edema, a flattened coarse facies, telangiectasias, and hepatosplenomegaly, but no dysostosis multiplex. The patient died 72 days postpartum. Excessive sialyloligosaccharides in urine, as well as vacuolation of lymphocytes and eosinophilic granulocytes in peripheral blood, were indicative of a lysosomal storage disease. In the patient's fibroblasts, both α-neuraminidase and β-galactosidase activities were severely reduced, and cathepsin A activity was <1% of control levels, confirming the biochemical diagnosis of galactosialidosis. However, in contrast to previously reported early-infantile cases, a normal amount of protective protein/cathepsin A mRNA was detected on Northern blots. This mutant transcript was translated into a precursor protein that was not processed into the mature enzyme and lacked both protective and catalytic activities.** © 1996 Wiley-Liss, Inc.

**KEY WORDS: galactosialidosis, cathepsin A, protective protein, lysosomal storage disease**

## INTRODUCTION

Galactosialidosis [d'Azzo et al., 1995] is an autosomal-recessive lysosomal storage disease caused by a primary defect of protective protein/cathepsin A (PPCA) [Wenger et al., 1978; d'Azzo et al., 1982; Galjart et al., 1988]. This enzyme has a specific serine carboxypeptidase/deamidase activity and, in addition, associates with lysosomal β-galactosidase and N-acetyl-α-neuraminidase, thereby regulating the intralysosomal stability and activity of the two glycosidases [reviewed in d'Azzo et al., 1995]. Deficiency of PPCA severely alters the activities of both β-galactosidase and neuraminidase, and this combined deficiency has been the hallmark of galactosialidosis. As in most other lysosomal storage diseases, galactosialidosis patients are clinically heterogeneous, having either a very severe early-onset form of the disease, mostly fatal at birth, or mild and slowly progressive late-onset types [d'Azzo et al., 1995]. Intermediate clinical manifestations of early- and late-infantile variants have also been observed in 2 patients [Sewell et al., 1987; Okada et al., 1983]. The spectrum of clinical manifestations correlates in part with differences in the expression level of PPCA mRNA [Galjart et al., 1988], as well as with the amount and quality of immunoprecipitated polypeptide [d'Azzo et al., 1982; Palmeri et al., 1986; Strisciuglio et al., 1988]. The isolation and characterization of human PPCA cDNA [Galjart et al., 1988] has enabled the identification of mutations associated with different clinical phenotypes [Zhou et al., 1991; Shimmoto et al., 1993; Suzuki et al., 1993].

Galactosialidosis is a rare lysosomal disorder. Thus far, most reported cases are juvenile/adult patients of Japanese origin [Suzuki et al., 1988; Takano et al., 1991]. Only a small number of Caucasian patients with the early-infantile form of the disease have been described [Kleijer et al., 1981; Gravel et al., 1979; Lowden et al., 1981; Carton et al., 1989]. Here, we report on the clinical, biochemical, and molecular characterization of the first Italian case.

## CLINICAL REPORT

The male patient was born to healthy, nonconsanguineous Italian parents (Fig. 1A). The first pregnancy

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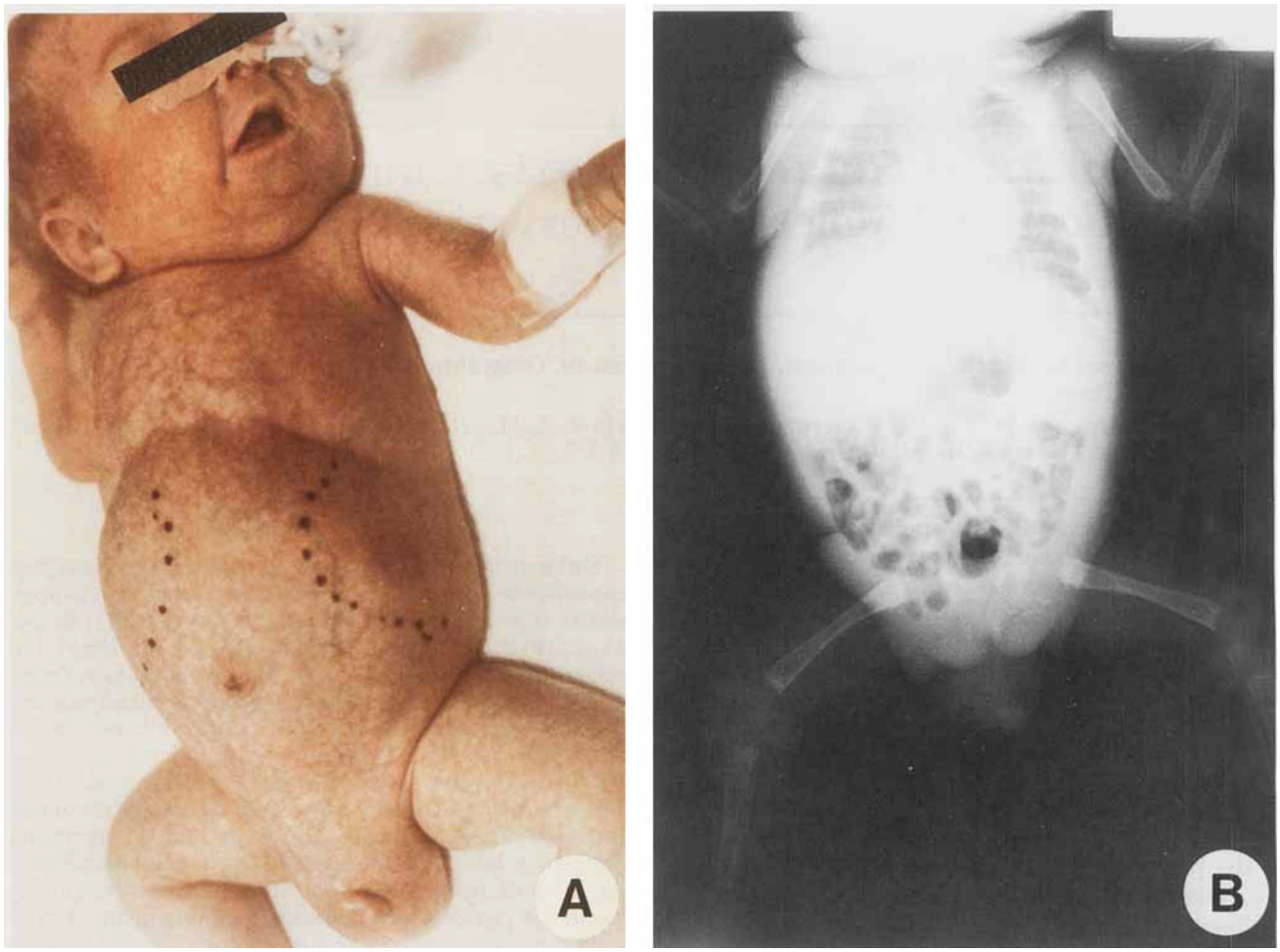
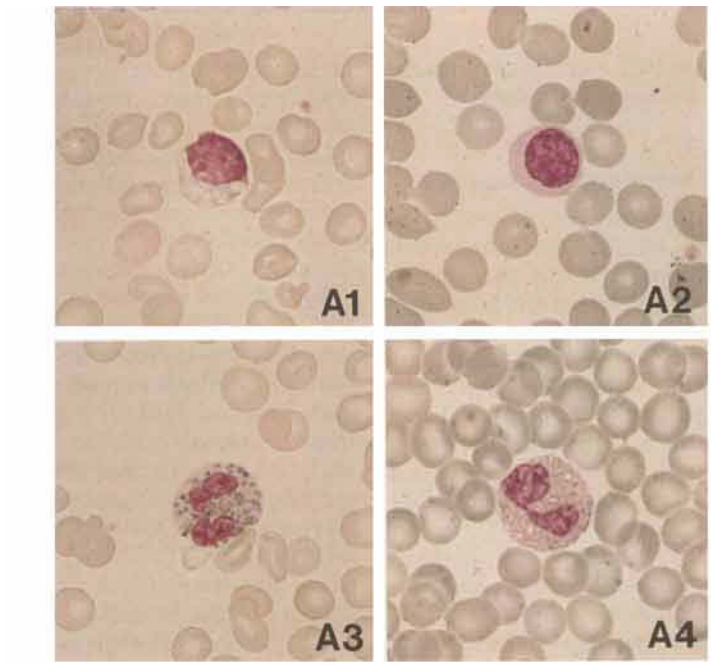


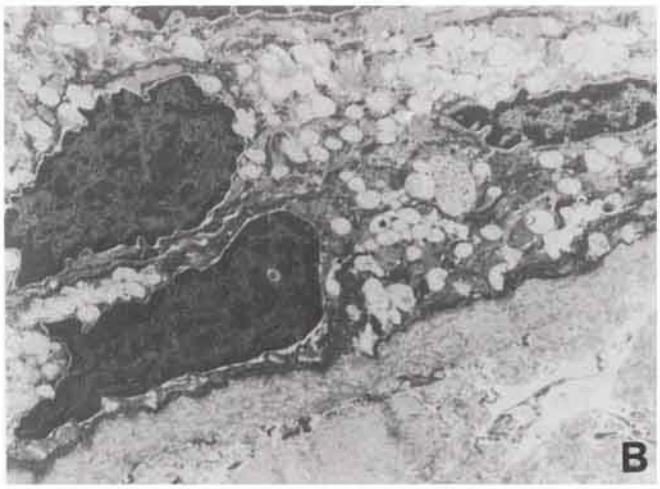

Fig. 1. **A:** Patient shows periorbital edema, coarse facies, hepatosplenomegaly, and widespread telangiectasias. **B:** Radiograph of skeleton shows a coarse trabecular pattern and thinning of the osseous cortex, without dysostosis multiplex.

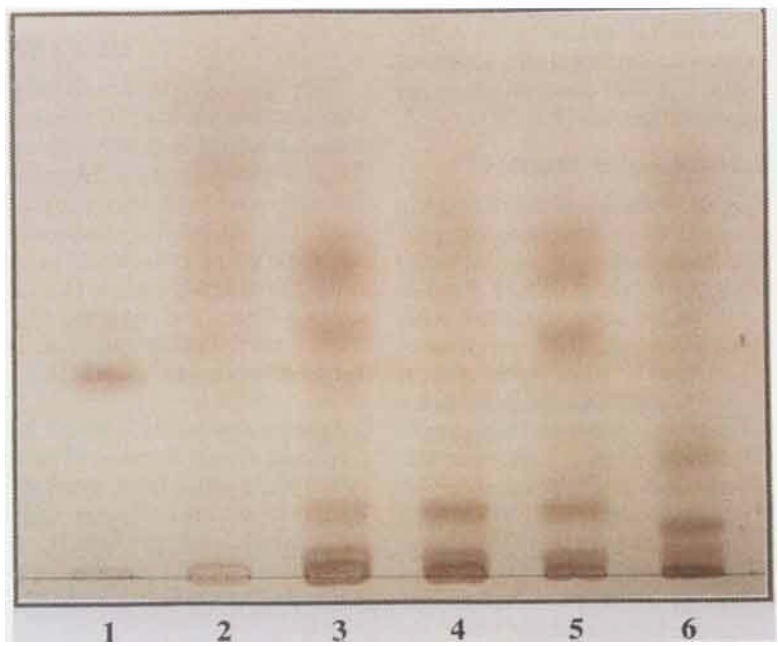
ended in first trimester miscarriage. This second pregnancy proceeded normally until week 24, when fetal hydrops was detected by ultrasound, showing no malformation. Tests for maternal infection, Rh and AB0 incompatibility, and chromosomal abnormalities proved negative. Cesarean delivery was performed at week 37 of gestation. At birth, weight was 2,090 g (<3rd centile), length 42 cm (<3rd centile), and head circumference 31 cm (<3rd centile). He had generalized hypotonia, massive edema, ascites, a flattened coarse facies, a depressed nasal bridge, apparently low-set ears, widespread telangiectasias, and marked hepatosplenomegaly. Cardiac arrhythmia was present. Transcranial ultrasonography showed slight enlargement of the ventricular system and periventricular calcifications at the roof of the right lateral ventricle. Radiographs of the skeleton showed a coarse trabecular pattern and thinning of the osseous cortex with a rickets-like appearance, but no dysostosis multiplex (Fig. 1B). Ophthalmologic examination, performed with difficulty due to the presence of periorbital edema, showed the presence of small, grayish discs. Chest radiograph showed cardiomegaly and a generalized granular pattern in the lungs. The patient's clinical condition deteriorated rapidly from severe respiratory distress, increasing unrelenting refractory edema, severe anemia, thrombocy-

Fig. 2. **A:** Peripheral blood smears from the patient and a normal individual, with May-Grünwald-Giemsa stain. **A1:** Abnormal lymphocyte with cytoplasmic vacuoles. **A2:** Normal lymphocyte. **A3:** Eosinophilic granulocyte with atypical features. Cytoplasm is unusually pale with coarse and gray-greenish stained granules. **A4:** Normal eosinophilic granulocyte. **B:** Electron micrograph of skin biopsy, showing fibroblasts and endothelial cells with some vacuoles containing reticular-granular material and numerous vacuoles with floccular material.

Fig. 3. Silica gel thin-layer chromatography of total urinary oligosaccharides. Our patient **(lane 3)** shows a pattern of excreted oligosaccharides similar to that observed in a late-infantile galactosialidosis patient **(lane 4)** and a sialidosis patient **(lane 5)**. A different pattern is detected in a case of type 1 $G_{M1}$-ganglisidosis **(lane 6)**. **Lane 1,** standard lactose. **Lane 2,** normal control.




Figs. 2 and 3

topenia, and seizures. Mechanical ventilation became necessary. The infant died 72 days postpartum.

## RESULTS

### Pathological and Laboratory Findings

Examination of the patient's peripheral blood, with May-Grünwald-Giemsa stain, showed the presence of a large number of lymphocytes with fine and coarse vacuoles (Fig. 2A1). Furthermore, eosinophilic granulocytes appeared pale, with coarse, grayish vacuoles (Fig. 2A3). Cells with the foamy appearance characteristic of lysosomal storage were seen in bone marrow smears. Electron microscopy of a skin biopsy specimen from the patient revealed numerous cytoplasmic membrane-enclosed vacuoles with heterogeneous and loosely-organized content in fibroblasts and endothelial cells (Fig. 2B).

The most important autopsy findings were: thickening and a jelly-like appearance of the subcutaneous adipose tissue; scattered, bilateral areas of pneumonitis; cardiomegaly with significant myocardial hypertrophy; cerebral edema with enlargement of the lateral ventricles; and periventricular calcifications. Histopathologic examination of paraffin sections showed fine cytoplasmic vacuoles in cells of most organs. The reticuloendothelial cells were the most severely affected. The alveolar cavities of the lungs were filled with large, macrophagic cells composed of foamy cytoplasm and small, round nuclei. The bronchial lumen and walls were infiltrated with polymorphonuclear cells. Fine vacuoles were present in the myocardial fibers. The hepatocytes showed severe, diffuse vacuolation, but the Kupffer's cells were particularly affected. Numerous vacuolated histiocytes were also present in the spleen. In the kidney, vacuolation of the glomerular endothelial and mesangial cells was noted. The cytoplasmic vacuoles were strongly colloidal, iron-positive, and faintly reactive with Alcian blue and periodic acid Schiff (PAS) staining.

Excretion of glycosaminoglycans was normal. Thin-layer chromatography of urinary oligosaccharides performed on silica-coated plates according to the methodology of Humbel and Collart [1975] showed elevated excretion of sialylated oligosaccharides (Fig. 3).

### Biochemical and Molecular Studies

Peripheral blood leukocytes from the patient and his parents were isolated according to the procedure of Kampine et al. [1966]. Skin fibroblasts were maintained in a 1:1 (v/v) mixture of Dulbecco's modified Eagle's medium and Ham's F10 medium supplemented with antibiotics and 10% fetal bovine serum. The activities of neuraminidase and β-galactosidase were measured in cell lysates of leukocytes and fibroblasts, as previously described [Galjaard, 1980], using commercially available fluorogenic substrates (Koch-Light Laboratories, Ltd., Colnbrook, UK). Cathepsin A activity was assayed according to the method of Galjart et al. [1991], using the acylated dipeptide Z-Phe-Ala as substrate (Bachem, PA). As shown in Table I, β-galactosidase activity was severely reduced in the patient's leukocytes, and a combined deficiency of neraminidase and β-galactosidase was demonstrated in cultured fibroblasts. Both enzyme activities were normal in the corresponding samples from the parents. Cathepsin A activity was <1% of control levels in the patient's fibroblasts. Clear heterozygous values were detected in fibroblasts from both parents. Together, these results confirmed the biochemical diagnosis of galactosialidosis.

In order to assess the molecular background of the PPCA deficiency in our patient, Northern blot analysis was performed. Total RNA was isolated from the patient's fibroblasts according to the procedure of Auffray and Rougeon [1980]. For comparison, RNA samples from a normal individual and a previously-described early-infantile patient [Galjart et al., 1988] were also included in this experiment. RNA samples were electrophoresed on a 0.8% agarose gel containing 2.2 M formaldehyde [Sambrook et al., 1989], blotted onto a nylon membrane (Zeta-Probe, Bio-Rad), and hybridized with a human protective-protein cDNA probe [Galjart et al., 1988; Sambrook et al., 1989]. As shown in Figure 4, in contrast to the other early-infantile case (Fig. 4, EI.1) who was totally devoid of PPCA mRNA, this patient (Fig. 4, EI.2) synthesized a 2-kb transcript comparable in amount to the control sample. However, biosynthetic labeling of the patient's fibroblasts and immunoprecipitation analysis demonstrated that this mutant mRNA directed the synthesis of a precursor protein of correct size and quantity that was not converted into the mature and active form of the enzyme (d'Azzo, manuscript in preparation). From these data we may infer that the mutation associated with this case of galactosialidosis impairs the transport of an otherwise normally-synthesized PPCA precursor to the lysosomes. Alternatively, but not exclusive of the first inference, the mutation may affect the intralysosomal stability of the protein. Consequently, its catalytic activity, as well as protective function towards β-galactosidase and neuraminidase, are both severely affected.

## DISCUSSION

The clinical course of early-infantile galactosialidosis is characterized by progressive and rapid deterioration leading to death within months after birth. This form of the disease is frequently associated with fetal hydrops and sometimes intrauterine death. In patient EI.2 (Fig. 4), fetal hydrops was detected by ultrasound at week 24 of gestation. In only one galactosialidosis patient reported earlier [Lowden et al., 1981] was fetal ascites diagnosed, at week 30 of gestation. However, in some cases, family histories have indicated the recurrence of perinatal death [Kleijer et al., 1979; Lowden et al., 1981].

Among the many defined causes of fetal or neonatal hydrops, those associated with lysosomal storage diseases have often been overlooked. In addition to galactosialidosis, other lysosomal disorders like Gaucher disease, $G_{M1}$-gangliosidosis, sialidosis, and mucopolisaccharidosis VII have been reported to provoke fetal hydrops [Van Maldergen et al., 1992]. Thus, this patho-

TABLE I. Enzyme Activities in Leukocytes and Fibroblasts From the Patient and His Parents*

| | Leukocytes | | | | Fibroblasts | | | |
|---|---|---|---|---|---|---|---|---|
| Enzyme | Patient | Mother | Father | N.V. | Patient | Mother | Father | N.V. |
| β-galactosidase | 3.42 | 137 | 140 | 100–270 | 53 | 430 | 579 | 427–995 |
| Neuraminidase | | | 1.5 | 0.59–1.1 | 2.2 | 63 | 49 | 42–117 |
| Cathepsin A | | | | | 0.5 | 173 | 181 | 243–486 |

* β-galactosidase and α-neuraminidase activities are expressed in nmol/hr/mg protein; cathepsin A is expressed in nmol/min/mg protein. N.V., normal values.

logical manifestation in early-infantile galactosialidosis patients, as well as in other patients with lysosomal storage diseases, may be more common than previously suspected, and the diagnosis may be missed because of the frequent occurrence of intrauterine or early postnatal death. Establishing the diagnosis by enzymatic assay is essential, not only for accurate genetic counseling, but also for prenatal diagnosis in subsequent pregnancies.

In the patient presented here, we suspected a lysosomal storage disease because of the presence of fetal hydrops, hepatosplenomegaly, coarse facies, and telangiectasias. Skeletal X-rays showed no dysostosis multiplex, but a coarse trabecular pattern and thinning of the osseous cortex were present. These findings have been reported in patients with neonatal ascites due to lysosomal storage diseases [Daneman et al., 1983], and they must be considered when the cause of hydrops or neonatal ascites is uncertain.

The presence of vacuolated lymphocytes in peripheral blood and foamy cells in the bone marrow is suggestive of a lysosomal storage disease. However, the numerous abnormal eosinophils detected in blood smears of the patient are an unusual feature that has been previously reported only in $G_{M1}$-gangliosidosis [Gitzelmann et al., 1985; Hansen and Graucob, 1985]. It is possible that this anomaly is specifically related to a primary or secondary deficiency of β-galactosidase [Donati et al., 1988]. If so, it would represent an additional diagnostic marker.

Histopathological examination of the patient's tissues demonstrated extensive cytoplasmic vacuolation of both epithelial cells and mesenchymal cells in all organs, although to variable degrees. The mechanism leading to the development of fetal hydrops is, however, unclear, although vacuolated cells were clearly evident in liver, kidney, and heart tissues.

In contrast to other cases of early-infantile galactosialidosis [Galjart et al., 1988], molecular studies in this patient showed a surprisingly normal level of PPCA mRNA, which directs the synthesis of a normal amount and size of the protective protein precursor. At this time, it is impossible to correlate the molecular data with the severe clinical phenotype. Further studies on a larger number of patients may clarify the biochemical and molecular bases of the severe early-infantile forms of galactosialidosis. The identification of the genetic lesion in the index patient may help to explain the effect of the mutation on the specific functions of PPCA which, in turn, may lead to a better understanding of the physiological role of this lysosomal enzyme in normal individuals.

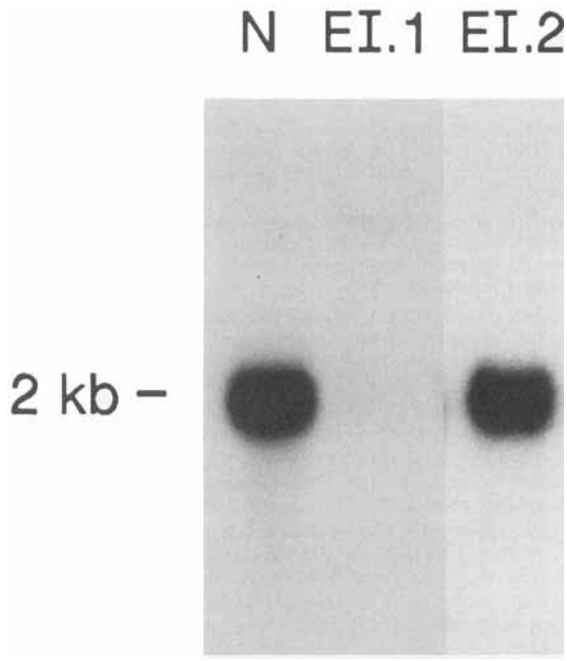



Fig. 4. Northern blot analysis of fibroblast total RNA. N, normal control; EI.1, early-infantile galactosialidosis patient without mRNA; EI.2, our early-infantile patient with normal amount of mRNA. Samples were fractionated on a formaldehyde agarose gel and probed with full-length PPCA cDNA. Size of PPCA transcript is indicated.

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